Title: Variations in photoprotective potential along gradients of leaf development and plant succession in subtropical forests under contrasting irradiances

Authors: Tai-Jie Zhang, Jin Zheng, Zheng-Chao Yu, Xiao-Qian Gu, Xing-Shan Tian, Chang-Lian Peng, Wah Soon Chow

PII: S0098-8472(17)30170-3
DOI: http://dx.doi.org/doi:10.1016/j.envexpbot.2017.07.016
Reference: EEB 3269

To appear in: *Environmental and Experimental Botany*

Received date: 3-5-2017
Revised date: 24-7-2017
Accepted date: 25-7-2017

Please cite this article as: Zhang, Tai-Jie, Zheng, Jin, Yu, Zheng-Chao, Gu, Xiao-Qian, Tian, Xing-Shan, Peng, Chang-Lian, Chow, Wah Soon, Variations in photoprotective potential along gradients of leaf development and plant succession in subtropical forests under contrasting irradiances.Environmental and Experimental Botany http://dx.doi.org/10.1016/j.envexpbot.2017.07.016

Variations in photoprotective potential along gradients of leaf development and plant succession in subtropical forests under contrasting irradiances

Tai-Jie Zhang[a,b], Jin Zheng[a], Zheng-Chao Yu[a], Xiao-Qian Gu[a], Xing-Shan Tian[b], Chang-Lian Peng[a,*], Wah Soon Chow[c,*]

[a] *Guangzhou Key Laboratory of Subtropical Biodiversity and Biomonitoring, Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, Guangzhou 510631, PR China*

[b] *Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, PR China*

[c] *Division of Plant Sciences, Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, Acton, Australian Capital Territory 2601, Australia*

[*] Corresponding authors; phone: +64 (0)3-479-7865, fax: +64 (0)3-479-7866, e-mail: pengchl@scib.ac.cn (C.-L. Peng); Fred.Chow@anu.edu.au (W. S. Chow)

Highlights

- We selected dominant trees of two successional stages from a subtropical forest.
- Photoprotective compounds were analyzed in various leaves of the trees.
- Photoprotective potential was inversely related to photosynthetic capacity.
- Late-successional trees had high levels of photoprotective potential.

ABSTRACT

The successful development of photosynthetic organs is the basis of plant growth and community development. To reveal photo-acclimation to high irradiance in tree species during the course of leaf development and plant succession of subtropical forests, photosynthetic efficiency and photoprotective compounds were analyzed in young and mature leaves of three mid-successional tree species (*Castanopsis fissa*, *Castanopsis chinensis* and *Schima superba*) and three late-successional tree species (*Machilus chinensis*, *Cryptocarya chinensis* and *Cryptocarya concinna*), grown in 100% full sunlight (FL) or 30% of FL (low light, LL). Young leaves of the two species groups exhibited lower chlorophyll (Chl) content, Rubisco content, net photosynthetic rate (P_n), carboxylation efficiency (*CE*), effective photochemical yield (Φ_{PSII}), photorespiratory electron flow (J_O), but higher dark respiration (R_d), and ratios of carotenoids/chlorophylls (Car/Chl), anthocyanins/chlorophylls (Anth/Chl), flavonoids/chlorophylls (Flav/Chl), phenols/chlorophylls (Phen/Chl) and total antioxidant capacity/chlorophylls (TAC/Chl) than those of mature leaves, regardless of growth irradiance. Young leaves of both species groups demonstrated a higher flexibility of Anth/Chl, Flav/Chl, Phen/Chl and TAC/Chl in response to different light conditions than mature leaves. Flav/Chl in young leaves of late-successional group was remarkably higher than that of mid-successional group under the same light conditions. There was a negative correlation between antioxidant-dependent photoprotective potential and photosynthetic efficiency in young and mature leaves of the six tree species grown under either FL or LL. Our results explain partial mechanisms that lie behind the replacement of communities in subtropical forests: highly integrated photoprotective potential allows young leaves of shade-tolerant late-successional species to develop smoothly into mature organs under high irradiance.

Key Words: community succession; flavonoids; irradiance; photoprotection; photosynthetic efficiency; young leaf

1. Introduction

Light-driven replacement of light-demanding trees by shade-tolerant trees is a major dynamic variation of evergreen broadleaf forests (Chazdon et al., 1996; Liu et al., 2007). In the course of community succession, light becomes increasingly scarce in the understory. Shade-tolerant late-succession species with a higher light-capturing capacity and a lower light-saturated photosynthetic capacity than earlier successional species can grow and regenerate seedlings in the shady habitat (Zhang et al., 2012; Zhu et al., 2013). However, when late-successional species are exposed to excess light, such as sunflecks in the understory, strong light in the canopy or canopy gaps, reduced photosynthetic efficiency can result in late-successional species encountering greater photo-oxidative stress than earlier successional species. Many late-successional species can acclimate to strong light by enhancing photoprotection mediated by xanthophylls in mature leaves (Zhang et al., 2012; Zhang et al., 2015). In regard to young leaves, however, little is known about the photoprotective tools they use to cope with excess light. Understanding the photoprotective variations between young and old leaves in dominant species of different successional stages should help to elucidate the mechanisms of subtropical forest succession.

Seedlings of late-successional species growing in the understory and canopy gaps can be exposed to sunflecks and bright sunlight, respectively (Canham, 1988; Chazdon, 1988). Moreover, some late-successional species can grow into canopy plants, thereby being exposed to full sunlight. In these cases, light intensities can be much higher than the light saturation point of photosynthesis in late-successional species. It is generally known that, in the presence of excess light, plants can absorb more photons than can be utilized, resulting in photoinhibitory or photooxidative damage to the photosystems (Murata et al., 2012; Goh et al., 2012). Due to the immaturity of chloroplasts, young leaves are more vulnerable to photoinhibition than mature leaves (Juvany et al., 2013). However, during long-term evolution, plants have developed several physical and biochemical strategies to mitigate the adverse effects of excess light. Physical strategies include leaf movement, chloroplast movement (Takahashi and Badger, 2011), pubescence (Liakopoulos et al., 2006), and the screening effect of non-photosynthetic

anthocyanin pigments (Feild et al., 2001; Hughes et al., 2005; Hughes and Smith, 2007). Biochemical strategies include the xanthophyll cycle (Müller et al., 2001), thermal energy dissipation (Demmig-Adams and Adams III, 2006), photorespiration (Voss et al., 2013; Silva et al., 2015) and enzymatic and non-enzymatic defense systems.

Non-enzymatic defense systems consist of low-molecular-weight antioxidants (LMWA), including ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids and phenolic compounds. Photoprotective activity facilitated by ascorbic acid, α-tocopherol, glutathione and carotenoids has been extensively studied in a great variety of plant species (Young, 1991; Smirnoff, 2000; Athar et al., 2008; Noctor et al., 2012; Dall'Osto et al., 2014). However, few studies have examined the photoprotection afforded by phenolic compounds other than anthocyanins, particularly in young leaves of woody plants. Instead, previous studies focused more on the functional role of phenolic compounds in young leaves in defense against insect herbivory than on protection against strong light (Hartley and Firn, 1989; Dudt and Shure, 1994; Rehman et al., 2012). Phenolic compounds, including anthocyanins, flavonoids and tannins, are secondary metabolites synthesized via the phenylpropanoid pathway. They not only rapidly neutralize reactive oxygen species (ROS) but also chelate transition metal ions, thereby mitigating the redox imbalance (Heim et al., 2002). It has been shown that the ROS-scavenging capacity of some flavonoids are four times greater than those of ascorbic acid and α-tocopherol (Rice-Evans et al., 1997; Wang et al., 1997). In addition to having antioxidative activity, anthocyanins as a special kind of flavonoids screen visible light. Leaves accumulating anthocyanins during development, giving young leaves their red color, is a common phenomenon. Besides the visible anthocyanins, other invisible phenolic compounds are also upregulated in leaves during development, and the increased levels are associated with high irradiance (Karageorgou and Manetas, 2006; Salgado et al., 2008; Zhang et al., 2016). In subtropical evergreen trees *Castanopsis fissa* (Champ. ex Benth.) Rehd. et Wils. and *Acmena acuminatissima* (Blume) Merr. et Perry, it has been found that the concentrations of total phenolic compounds are dozens of times that of anthocyanins (Zhang et al. 2016; Zhu et al. 2017). This suggests that total phenolic compounds as antioxidants can provide much greater

photoprotection for young leaves than anthocyanins.

The Dinghu Mountain National Natural Reserve (DMNNR), which is situated in the low subtropical zone, in the middle of Guangdong Province, China, has a complete successional series of forest communities, from the early coniferous forest stage to the latest-successional broad-leaved evergreen forest stage (Peng and Wang, 1995). The light-loving trees *Castanopsis fissa* (Champ. ex Benth.) Rehd. et Wils., *Castanopsis chinensis* (Spreng.) Hance. and *Schima superba* Gardn. et Champ. are dominant species in the mid-successional stage, and the shade-tolerant trees *Machilus chinensis* (Champ. ex Benth.) Hemsl., *Cryptocarya chinensis* (Hance.) Hemsl. and *Cryptocarya concinna* Hance. are dominant species in the late-successional stage (Zhu et al. 2013). All the six species are evergreen canopy trees, which can produce new leaves all year round, particularly the late-successional species. Young leaves of the six species can appear red under different conditions due to the accumulation of anthocyanins: *C. fissa* and *C. concinna* tend to be red all year round, *C. chinensis* and *S. superba* tend to red in the summer under high light, whereas *M. chinensis* and *C. chinensis* tend to be red in the winter. Despite all this, anthocyanins are only the tip of the iceberg of the total phenolic pool in young leaves. We used these six dominant tree species of two different successional stages for this study. Based on pot experiments, phenols, flavonoids, anthocyanins, total antioxidant capacity, photosynthetic characteristics and allocation of photosynthetic electron flow were determined in young and mature leaves of the six tree species grown in contrasting irradiances. We hypothesized that (1) phenolic compounds as an important aspect of photoprotection for young leaves is more responsive to irradiance than other photoprotective tools; young leaves of late-successional species have a higher content of phenolic compounds and the resulting integrated photoprotective potential is greater than that of mid-successional species, due to an increased requirement for photoprotection; (2) photoprotective relationship between young and mature leaves is similar to the relationship in mature leaves between mid- and late-successional species.

2. Materials and methods

2.1. Plant material and experimental conditions

The saplings (height 30-50 cm) of three mid-successional dominant tree species, *C. fissa*, *C. chinensis* and *S. superba*, and three late-successional dominant tree species, *M. chinensis*, *C. chinensis* and *C. concinna* were collected from the DMNNR (23°09′21″-23°11′30″ N, 112°30′39″-112°33′41″ E) in March 2015, and were pot-grown in the biological garden (23°8′22.39"N, 113°20′59.05"E) of South China Normal University (Guangzhou, China). The experimental site has a subtropical humid monsoon climate, with annual mean air temperature of 22.8°C and annual mean rainfall of 1,736 mm. The plastic pots (40 cm diameter and 32 cm depth) used for culturing the plants were filled with a clay loam soil mixed with peat soil (3:1 in volume). After transplanting, the saplings were allowed to re-grow for two months under reasonable management, and then the plants were divided into two groups (each group contained twelve potted plants per species), grown under 100% full sunlight (FL) and 30% of full sunlight (low light, LL) conditions. The LL conditions were provided by two layers of black nylon shading net, which simulated the understory environments of subtropical forests. The relative irradiance (%) in LL was estimated by the method as previously described (Zhu et al., 2016). The plants were adequately fertilized with compound fertilizer once monthly and watered as required about once a day in summer and three times a week in the other seasons. From July to August in 2016, two young leaves, designated as the first (1st) and second (2nd) young leaves, and the fully mature leaves (ML) were selected from 4-5 individuals of each tree species for physiological and biochemical analyses. Leaf length, width and relative maturity are shown in Table 1.

2.2 Chlorophyll determination

Three 10-mm-diameter leaf discs were punched from leaves, ground with liquid nitrogen, submerged in 4 mL of 80% acetone, and then placed at 4°C in the dark overnight for chlorophyll extraction. After blending and centrifugation at 8,000 × *g* for 10 min, chlorophyll and carotenoid concentrations in the extract were spectrophotometrically assessed according to Wellburn (1994).

2.3 Gas exchange measurements

A portable infrared gas analyser Li-6400 (LI-COR, Inc., USA) was used to measure gas exchange parameters of various leaves of the six species in the morning

(8:30-12:00). Photosynthetically active radiation was emitted from a red and blue (9:1) LED light source, which was integrated into the LI-6400 leaf measurement chamber. During measurements of net photosynthetic rate (P_n) at the saturating photosynthetic photon flux density (PPFD) 800 μmol m^{-2} s^{-1}, and of dark respiration rate (R_d) at PPFD 0 μmol m^{-2} s^{-1}, CO_2 concentrations provided in the leaf chamber were 400 μmol mol^{-1}, leaf temperature was ~30°C and humidity was 60-70%. After reaching steady state in the device, gas exchange parameters were manually recorded and saved. Photosynthetic carbon dioxide (A/C_i) response curves were measured on leaves of the six tree species at PPFD 800 μmol m^{-2} s^{-1} using ten CO_2-concentraion steps (400, 200, 20, 80, 150, 200, 500, 800, 1200 and 1600 μmol mol^{-1}). The waiting times were 90-150 s for each CO_2 concentration. Leaves were fully adapted to the PPFD 800 μmol m^{-2} s^{-1} for 5 min before measurement of A/C_i response curve. During measurement, the device automatically recorded and stored net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (G_s) and transpiration rate (T_r). Carboxylation efficiency (*CE*) was derived from the slop based on a linear regression of A/C_i response curve at $C_i <$ 200 μmol mol^{-1}.

2.4 Chlorophyll fluorescence analysis

A portable pulse-amplitude-modulated (PAM) fluorometer PAM-2100 (Walz, Germany) was used to determine chlorophyll fluorescence on the leaves on which gas exchange parameters had been measured before. Leaves of the six tree species were continually illuminated for ~5 min at the PPFD 800 μmol m^{-2} s^{-1}; steady-state chlorophyll fluorescence (F_s) and maximal fluorescence (F_m') in the light-adapted state were then determined. The effective quantum yield of PSII (Φ_{PSII}) was calculated as: $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty et al., 1989). The total photosynthetic electron flow (J_T) were calculated as $J_T = \Phi_{PSII} \times$ PPFD $\times$ 0.85 $\times$ 0.5, where the coefficient 0.85 was the assumed average leaf absorptance and the coefficient 0.5 is the assumed proportion of absorbed photon allocated to PSII (Krall and Edwards, 1992). Allocation of J_T to Rubisco-mediated carboxylation (J_C) and oxygenation (J_O) were calculated as (Valentini et al., 1995): $J_C = 1/3(J_T + 8(P_n + R_d))$; $J_O = 2/3(J_T - 4(P_n + R_d))$, where P_n

and R_d are the net photosynthetic rate and dark respiration rate, respectively.

Non-photochemical quenching (NPQ) was measured by using a chlorophyll fluorescence imaging system (Technologica, UK). Young and mature leaves were detached from five individuals of each species at the morning (08:30), which experienced PPFDs less than 150 μmol m^{-2} s^{-1} in that day. After dark-adapted at 26°C ambient temperature at least for 30 min, the minimum fluorescence (F_o) and the maximum fluorescence (F_m) were then measured on leaves by using a 6,000 μmol m^{-2} s^{-1} saturating pulse. Similarly, light-adapted fluorescence (F) and maximum fluorescence (F_m') were recorded after leaves were exposed to a 800 μmol m^{-2} s^{-1} actinic light for 5 min. NPQ was calculated as: NPQ = $(F_m/F_m') - 1$ (Bilger and Björkman 1990).

2.5 Rubisco estimation

Six leaf discs (9 mm in diameter) were homogenized in 0.8 mL of 60 mM Tris-HCl (pH 7.8) buffer, which contained 0.1% (w/v) NaCl, 2% (v/v) glycerol and 5% (w/v) polyvinylpyrrolidone (PVP). After centrifugation at 12,000 × g and 4°C for 15 min, total protein content in the supernatant was determined by the Bradford (1976) method, and bovine serum albumen (BSA) was used to construct the calibration curve. 0.1 mL supernatant was mixed with an equal volume of protein loading buffer, incubated at 100°C for 5 min and stored at 4°C for Rubisco analysis. Rubisco protein was separated by SDS-PAGE method as previous described by Zhang et al. (2016). The large and small subunit of Rubisco were easily recognized according to molecular weight and abundance. For calculation of the content of Rubisco protein, each SDS-PAGE gel contained a 10-400 μg mL^{-1} series of BSA. Relative volume of Rubisco and BSA bands were analyzed by using a TotalLab Quant software (TotalLab, UK). Rubisco content based on unit leaf area was calculated through a calibration curve of BSA band volume versus concentration.

2.6 Anthocyanin determination

Four leaf discs (10 mm in diameter) were submerged in 4 mL of methanol:HCl

(99:1, v/v) at 4°C in the dark for 24 h for extraction of anthocyanin pigments. Subsequently, 4-mL chloroform and 1.5 mL deionized water were added to the extract for removal of chlorophylls. After blending, anthocyanins were dissolved in the upper layer of solution, whereas chlorophylls were dissolved in the lower solution. The volume of the upper layer solution was determined, and its absorbance was measured at 530 nm against methanol:HCl (99:1, v/v) as a blank. Canidin-3-O-glucoside (5-200 μM) was used as the standard for calibration of anthocyanin concentration.

2.7 Determination of total content of flavonoids and phenols

Total flavonoids and phenols were extracted from two 10-mm-diameter leaf discs in 1.5 mL of 95% methanol at 4°C for 24 h. Total flavonoids were determined by using the method described by Heimler et al. (2005) with a slight modification. Briefly, 0.2 mL of 5% $NaNO_2$, 0.3 mL of freshly prepared 10% $AlCl_3$, 1 mL of freshly prepared 1 M NaOH and 1.5 mL of deionized water was successively added into 0.5 mL of 10-fold diluted sample. After standing at room temperature for 5 min, the absorbance of the mixture at 510 nm was determined by using a spectrophotometer (UV2450, Shimadzu, Kyoto, Japan). Total content of flavonoids was expressed as catechin equivalents (μmol cm^{-2} area).

Total content of phenols was determined by the Folin–Ciocalteu method according to a previously reported procedure (Ainsworth and Gillespie, 2007). Briefly, 1 mL of 10% Folin–Ciocalteu and 2 mL of 0.7 M Na_2CO_3 was added to 0.5 mL of 10-fold diluted sample. After standing for 5 min, the absorbance of the mixture was measured at 760 nm. Phenol concentration was calculated according a Gallic acid (50-250 μM) calibration curve.

2.8 Determination of total antioxidant capacity (TAC)

Total antioxidants were extracted in the same way as extraction of flavonoids and phenols. TAC of the six tree species was measured by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay, according to a previously reported procedure (Saha et al., 2008). Briefly, 3 mL of 100 μM DPPH solution (fleshly prepared in 95% methanol) was added to 10 μL of sample. After standing for 5 min, the decrease in absorbance at 517 nm was

measured against 95% methanol as a blank. The TAC was calculated through a calibration curve established using 20-100 μM DPPH, and finally expressed as μmol DPPH per unit area.

2.9 Data analysis

Differences in physiological and biochemical traits between the two successional groups under contrasting light were tested with one-way ANOVA by using IBM SPSS Statistics 19.0 software (IBM, NY, USA). Tukey test was used for post hoc analysis at the 0.05 level. Relationship between Flav/Chl, Phen/Chl and TAC/Chl were analyzed using a linear regression analysis by using Sigmaplot 12.5 software (Systat Software Inc., USA). Multivariate associations of the 15 leaf traits (Supporting Information Table S1) were analyzed with a principal component analysis (PCA) by using IBM SPSS Statistics 19.0 software (IBM, NY, USA), using the mean values of the traits without transformation. To determine photoprotective relationships between young and mature leaves under contrasting irradiances, secondary PCA was further performed on photoprotective traits (Anth/Chl, Flav/Chl, Phen/Chl, TAC/Chl and Car/Chl) and photosynthetically associated traits (leaf length, Chl, Rubisco, P_n, *CE*, Φ_{PSII}) separately. Mean factor loadings of leaves of the six species at 3 different developmental stages between the photoprotective and photosynthetic PCA axes were analyzed with linear regression.

3. Results

3.1 Leaf appearances and photosynthetic pigments

Leaves of the six trees species grown under FL conditions manifested distinctly different appearances from those grown under LL (Fig. 1). Both young and mature leaves exposed to LL were greener than the corresponding leaves exposed to FL. In LL, all the six tree species tended to have bigger mature leaf size than those in FL. Under FL, young leaves of mid-successional species *C. fissa*, *C. chinensis* and *S. superba* appeared red. By contrast, the redness was reduced in young leaves of *C. fissa* and *C. chinensis* under LL, and disappeared in young leaves of *S. superba*. No redness was found in young leaves of late-successional species *M. chinensis* and *C. chinensis* under either FL or LL conditions. However, young leaves of *C. concinna* were red under both

light conditions.

Total chlorophyll (Chl) content continually increased in older versus younger leaves (Fig. 2A), coinciding with leaf appearances (Fig. 1). The Chl content in young leaves of the mid-successional species group exposed to LL was higher than that in young leaves exposed to FL, and higher than that of the corresponding leaves of late-successional species exposed to either FL or LL (P<0.05). The Chl content in young leaves of late-successional species group exposed to LL was also slightly higher than in those exposed to FL, though the difference was not statistically significant. Regardless of light conditions, young leaves of both species groups had a lower Chl *a*/*b* ratio, but a greater Car/Chl ratio than mature leaves (Fig. 2B, C). Young and mature leaves of both species groups grown in FL generally had higher ratios of Chl *a*/*b* and Car/Chl than those grown in LL. The Chl *a*/*b* ratio was comparable in young or mature leaves between mid- and late-successional species groups at the same growth irradiance. However, the Car/Chl ratio in young or mature leaves of late-successional group was higher than that of mid-successional group under the same light conditions (Fig. 2C).

3.2 Photosynthetic characteristics and electron transport

Reduced Chl content per unit leaf area limited the capture of light energy, resulting in the net photosynthetic rate (P_n) being dramatically lower in young than mature leaves of both species groups (Fig. 2D). Under both FL and LL, there were no significant differences in P_n in the 1st or 2nd young leaves between the mid- and late-successional groups. P_n in mature leaves of mid-successional group was greater than that of the late-successional group. Compared with FL conditions, P_n was slightly decreased in mature leaves of both mid- and late-successional group under LL conditions. Being matched with P_n, the effective photochemical yield of PSII (Φ_{PSII}) in young leaves of the two successional groups were all lower than those of mature leaves (Fig. 2E). But unlike P_n, Φ_{PSII} in young leaves were significantly lower in late-successional group than in mid-successional group at the same growth irradiance (P<0.05) (Fig. 2E). Non-photochemical quenching (NPQ) showed a similar tendency as Chl *a*/*b* and Car/Chl in FL-exposed leaves versus LL-exposed leaves of the two successional groups (Fig. 2F). Both successional groups have higher NPQ in FL than

in LL in leaf at the same life stage. Compared with mid-successional group, the late-successional group evidently had lower NPQ.

Along a gradient of leaf development, Rubisco content and carboxylation efficiency (*CE*) in the two successional groups under FL or LL had the same trends of change as did P_n (Fig. 3A, B). On the contrary, dark respiration (R_d) in young leaves of both species groups was greater than in mature leaves (Fig. 3C). Young leaves of mid-successional group had higher R_d than those of late-successional group.

Light energy is absorbed by leaves, converted into electron flow, and finally utilized by Rubisco-mediated carboxylation (J_O) and oxygenation (J_C). During the course of leaf development, the varying tendencies of J_O and J_C in the two successional groups were all completely consistent with the trend for Φ_{PSII} (Fig. 2E; Fig. 3D, E). Since J_O and J_C had the same changing patterns, no significant differences were found in the proportion of photosynthetic electron transport to photorespiration (J_O/J_T) in young or mature leaves between the two successional groups under the same growth irradiances, or in the same successional group grown under contrasting irradiances (Fig. 3F).

3.3 Phenolic antioxidants and total antioxidant capacity

Young leaves of both species groups from the mid- and late-successional stages had higher ratios of anthocyanins/chlorophylls (Anth/Chl), flavonoids/chlorophylls (Flav/Chl), phenols/chlorophylls (Phen/Chl), and total antioxidant capacity/chlorophylls (TAC/Chl) than the corresponding ratios of mature leaves under the same light conditions (Fig. 4). In addition, young and mature leaves of mid- and late-successional group grown in FL had higher ratios of Anth/Chl, Flav/Chl, Phen/Chl and TAC/Chl than those grown in LL. No significant differences were observed in the ratios of Anth/Chl, Phen/Chl and TAC/Chl in young leaves between the two successional groups in the same growth irradiance. By contrast, there were dramatic differences in Flav/Chl ratio in young leaves between the two successional groups in the same growth irradiance. To reveal the contribution of total flavonoids and phenols to total antioxidant capacity, TAC/Chl is plotted against Flav/Chl and Phen/Chl in young and mature leaves of the six tree species (Fig. 4E, F). TAC/Chl in young and

mature leaves of six tree species showed positive correlations with Flav/Chl or Phen/Chl (P<0.001). From the linear regression, it is seen that TAC/Chl depended on both Flav/Chl and Phen/Chl (Fig. 4E, F).

3.4 Principal component analysis

A principal component analysis (PCA) was conducted on 15 functional traits in young and mature leaves of the six tree species (Fig. 5). The first two principal components (PCs) captured 68.1% of the variance, with 54.1% in the first axis, PC1 (Fig. 5A). PC1 showed strong positive loadings for leaf length, Chl, Rubisco content, P_n, CE, Φ_{PSII}, whereas Anth/Chl, Flav/Chl, Phen/Chl, TAC/Chl and Car/Chl were loaded at the negative end. Since leaf length, Chl, Rubisco, P_n, CE, Φ_{PSII} reflect plant photosynthetic efficiency, and Anth/Chl, Flav/Chl, Phen/Chl, TAC/Chl and Car/Chl reveal plant photoprotective potential, PC1 could be construed as a photosynthetic efficiency-photoprotective potential factor. The second axis, PC2, explained 14.0% of the variation, and was primarily structured by Chl a/b. The Chl a/b ratio reflects acclimation of plant leaves to different levels of irradiance, so that PC2 could simply considered to be an irradiance factor. After mean loadings on first and second PC axes were calculated for 1st and 2nd young leaves and mature leaves of six species categorized into mid- and late-successional groups (Fig. 5B), it is found that mature leaves of both groups exhibited a higher photosynthetic efficiency and a lower photoprotective potential than those of young leaves.

Since positive correlations were found among leaf length, Chl, Rubisco, P_n, CE, Φ_{PSII} (Tab. S1; Fig. 5A), secondary PCA of these 6 functional traits extracted a 'photosynthetic efficiency' PC that explained 81.8% of the variance (Fig. 5C). Similarly, secondary PCA of Anth/Chl, Flav/Chl, Phen/Chl, TAC/Chl and Car/Chl obtained an antioxidant-dependent 'photoprotective potential' PC that explained 78.5% of the variance (Fig. 5D). Significant correlations were found between the first PCA axe of photoprotective potential and that of photosynthetic efficiency in young and mature leaves of the six tree species from different successional stages under both FL and LL conditions (Fig. 5E). This photoprotective relationship integrated the variation tendency of leaf development and plant succession, indicating that photoprotection along a gradient of leaf development is consistent with that along a gradient of plant

succession. Moreover, the differences in photoprotective potential between FL and LL conditions increased as photosynthetic efficiency decreased. Thus, photoprotective potential in young leaves with reduced photosynthetic efficiency were more responsive to growth irradiance. This had led to the absolute value of the regression slope (2.12) in leaves exposed to FL being greater than that (1.32) in leaves exposed to LL.

4. Discussion

Mature leaves of late-successional species have been demonstrated to have a lower photosynthetic efficiency than earlier successional species in subtropical forests (Zhu et al., 2013; Zhang et al., 2015; Zhu et al., 2016). This study further showed that young leaves of late-successional species also had lower photosynthetic efficiency than those of mid-successional species in both FL and LL (particularly in LL), as reflected by Chl content, Rubisco content, P_n, CE, Φ_{PSII} and J_C (Figs. 2 and 3). Mature leaves of late-successional species in evergreen and deciduous forests have been shown to be more vulnerable to photoinhibition to than that of earlier successional species (Kitao et al. 2000; Favaretto et al. 2011). Similar to the case of mature leaves, young leaves of late-successional species should be also more vulnerable to photoinhibition than young leaves of mid-successional species. For seedlings of late-successional species growing in the understory, sunflecks and sun patches due to canopy gaps are the source of excess light (Kitao et al. 2000; Kitao et al. 2006). After late-successional species grow into the canopy, they are exposed to full sunlight which is also a source of excess light. To avoid the harmful effects of excess light, both young and mature leaves of late-successional species improved their photoprotective capacity by optimizing the ratios of antioxidants to chlorophylls (Fig. 4).

NPQ, antioxidants such as carotenoids, flavonoids and phenols, and photorespiration are important tools for leaves to mitigate photoinhibition of photosynthesis (Agati and Tattini, 2010; Takahashi and Badger, 2011; Jahns and Holzwarth, 2012). In both FL and LL conditions, young leaves of the two kinds of species had higher NPQ, Car/Chl, Anth/Chl, Flav/Chl, Phen/Chl and TAC/Chl than that of mature leaves. This explains the fact that young leaves have increased requirement

of photoprotection due to their immature chloroplasts. Since young leaves are at the most vulnerable life stage for insect attack, another reason for the up-regulation of secondary phenolic metabolites in young leaves is to improve herbivory defense (Dudt and Shure, 1994). However, the amplitude of variation of Anth/Chl, Flav/Chl, Phen/Chl and TAC/Chl in young leaves of the two kinds of species between the two growth irradiances was obviously greater than that NPQ and Car/Chl, indicating that regulation of secondary phenolic metabolites in young leaves may be the key strategy allowing them to acclimate to high light. Compared with mid-successional species, young leaves of late-successional species in the same growth irradiance had comparable ratios of Anth/Chl, Phen/Chl and TAC/Chl, but a greater ratio of Flav/Chl. By contrast, young leaves of the late-successional species had lower NPQ than those of mid-successional species. This suggests that secondary phenolic metabolites are more important than NPQ for the improvement of photoprotective capacity in young leaves of late-successional species. Among the secondary phenolic metabolites, the most striking differences between young leaves of mid- and late-successional species was the difference in flavonoids per unit Chl (Fig. 3), and per unit area (Fig. S1). Flavonoids are selected as an important part of the strategy to enable young leaves of late-successional species to acclimate to high light, possibly because flavonoids have a higher free radicals scavenging activity than other antioxidants, such as ascorbic acid and tocopherol (Rice-Evans et al. 1997; Wang et al., 1997). In addition to enhancing free radicals scavenging activity, the large pool of flavonoids in young leaves of late-successional species may also facilitate the accumulation of anthocyanins under stressful conditions, which can shade the photosynthetic apparatus (Hughes et al., 2007; Zhang et al., 2016). Moreover, since flavonoids are involved in regulation of auxin transport and plant organ development (Agati and Tattini, 2010), FL-exposed mature leaves of the six tree species, with a smaller size than those LL-exposed mature leaves, may be regulated by increased flavonoids during development.

Anthocyanins are a special type of flavonoids, which can afford photoprotection not just by scavenging of reactive oxygen species (ROS), but more importantly through functioning as a light attenuator (Hughes et al., 2005; Tucić et al., 2009; Zhang et al.,

2010; Zhang et al., 2016). Redness of young leaves due to anthocyanins is a widespread phenomenon in tropical and subtropical forests. In this study, Anth/Chl was, on average, roughly the same in the young leaves between the two successional species groups (Fig. 4A). Anthocyanin content per unit area in young leaves of mid-successional species was less than that of mid-successional species (Fig. S1). This is not consistent with previous results in which late-successional species had higher amounts of anthocyanins than in mid-successional species (Zhu et al., 2016). This is likely because the studies were conducted at different times of the year: this study was performed from July to August, while, that of Zhu et al. (2016) was performed from September to October. Indeed, observation of potted plants and plants in the field showed that young leaves of late-successional species tend to be redder in autumn and winter than other seasons of the year.

Though photorespiration is also involved in photoprotection (Jiang et al. 2006; Bai et al. 2008; Silva et al., 2015), young leaves of late-successional species had lower photorespiration than mid-successional species under the same growth irradiance, as indicated by J_O (Fig. 3D). In addition, in terms of the proportion of photosynthetic electron transport devoted to photorespiration (J_O/J_T), no remarkable difference in young leaves was found between the two successional groups (Fig. 2F). Therefore, photorespiration did not contribute to improvement of photoprotective capacity in leaves of late-successional species relative to mid-successional species, possibly due to the scarcity of Rubisco (Fig. 3A).

In conclusion, along a gradient of leaf development, Chl content, Rubisco content, P_n, Φ_{PSII} and *CE* increased in both mid- and late-successional species, whereas Anth/Chl, Flav/Chl, Phen/Chl, TAC/Chl and Car/Chl decreased. By contrast, these two groups of parameters showed an opposite tendency along the community successional axis. The combined effect and inter-coordination of anthocyanins, flavonoids, phenols and carotenoids afforded a stronger integrated photoprotective potential in the young than mature leaves, and in the late- than mid-successional species. Photoprotective potential was negatively correlated with photosynthetic efficiency in all the young and leaves in both FL and LL conditions. Another way to interpret this negative correlation is that as the photosynthetic efficiency was lowered, there was a greater need for

increased photoprotective potential; similarly, at a given photosynthetic efficiency, the need for photoprotective potential was greater in FL than in LL.

The shade tolerance of late-successional species requires them to assemble a photosynthetic apparatus that can effectively produce enough organic carbon for plant growth in low light conditions. To meet this need, late-successional species make a compromise in photosynthetic efficiency. Due to reduced photosynthetic efficiency, late-successional species are more vulnerable to photooxidative damage than mid-successional species (Kitao et al. 2000). High responsive thermal dissipation may enable mature leaves of late-successional tree species to acclimate to high light conditions (Zhang et al., 2015). In autumn, young leaves of late-successional species accumulate higher amounts of anthocyanins to guarantee leaf development (Zhu et al., 2016). Further, this study indicates that young leaves of late-successional species have a higher antioxidant-dependent integrated photoprotective potential than those of earlier successional species, thus allowing young leaves to develop smoothly into mature productive organs under strong sunlight in summer. This explains partial mechanisms that lie behind the replacement of communities of subtropical forests and enriches the understanding of the use of photoprotective tools in young and mature leaves during the course of plant succession of subtropical forests.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (31570398, 31270287). The study was also supported by the key programme of Guangdong Province Natural Science Foundation (2015A030311023).

References

Agati, G., Tattini, M., 2010. Multiple functional roles of flavonoids in photoprotection. New Phytol. 186, 786-793.

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2, 875-877.

Athar, H.-u.-R., Khan, A., Ashraf, M., 2008. Exogenously applied ascorbic acid

alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot. 63, 224-231.

Bai, J., Xu, D., Kang, H., Chen, K., Wang, G., 2008. Photoprotective function of photorespiration in *Reaumuria soongorica* during different levels of drought stress in natural high irradiance. Photosynthetica 46, 232-237.

Bilger, W., Björkman, O., 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynthesis Res. 25, 173-185.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analy. Biochem. 72, 248-254.

Canham, C.D., 1988. Growth and canopy architecture of shade- tolerant trees: response to canopy gaps. Ecology 69, 786-795.

Chazdon, R.L., 1988. Sunflecks and their importance to forest understorey plants. Adv. Ecol. Res. 18, 1-63.

Chazdon, R.L., Pearcy, R.W., Lee, D.W., Fetcher, N., 1996. Photosynthetic responses of tropical forest plants to contrasting light environments, in: Mulkey, S.S., Chazdon, R.L., Smith, A.P. (Eds.), Trop. Forest Plant Ecophysiol. Springer US, Boston, MA, pp 5-55.

Dall'Osto, L., Bassi, R., Ruban, A., 2014. Photoprotective mechanisms: carotenoids, in: Theg, S.M., Wollman, F.-A. (Eds.), Plastid Biology. Springer New York, New York, NY, pp 393-435.

Demmig-Adams, B., Adams III, W.W.A. 2006. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol. 172, 11-21.

Dudt, J.F., Shure, D.J., 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. Ecology 75, 86-98.

Favaretto, V.F., Martinez, C.A., Soriani, H.H., Furriel, R.P.M., 2011. Differential responses of antioxidant enzymes in pioneer and late-successional tropical tree

species grown under sun and shade conditions. Environ. Exp. Bot. 70, 20-28.

Feild, T.S., Lee, D.W., Holbrook, N.M., 2001. Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol. 127, 566-574.

Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophy. Acta 990, 87-92.

Goh, C.-H., Ko, S.-M., Koh, S., Kim, Y.-J., Bae, H.-J., 2012. Photosynthesis and environments: photoinhibition and repair mechanisms in plants. J. Plant Biol. 55, 93-101.

Hartley, S.E., Firn, R.D., 1989. Phenolic biosynthesis, leaf damage, and insect herbivory in birch (*Betula pendula*). J. Chem. Ecol. 15, 275-283.

Heim, K.E., Tagliaferro, A.R., Bobilya, D.J., 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13, 572-584.

Heimler, D., Vignolini, P., Dini, M.G., Romani, A., 2005. Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L. dry beans. J. Agric. Food Chem. 53, 3053-3056.

Hughes, N., Neufeld, H., Burkey, K., 2005. Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata*. New Phytol. 168, 575-587.

Hughes, N.M., Morley, C.B., Smith, W.K., 2007. Coordination of anthocyanin decline and photosynthetic maturation in juvenile leaves of three deciduous tree species. New Phytol. 175, 675-685.

Hughes, N.M., Smith, W.K., 2007. Attenuation of incident light in *Galax urceolata* (Diapensiaceae): concerted influence of adaxial and abaxial anthocyanic layers on photoprotection. Am. J. Bot. 94, 784-790.

Jahns, P., Holzwarth, A.R., 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim. Biophy. Acta 1817, 182-193.

Jiang, C.-D., Gao, H.-Y., Zou, Q., Jiang, G.-M., Li, L.-H., 2006. Leaf orientation, photorespiration and xanthophyll cycle protect young soybean leaves against

high irradiance in field. Environ. Exp. Bot. 55, 87-96.

Juvany, M., Müller, M., Munné-Bosch, S., 2013. Photo-oxidative stress in emerging and senescing leaves: a mirror image? J. Exp. Bot. 64, 3087-3098.

Karageorgou, P., Manetas, Y., 2006. The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. Tree Physiol. 26, 613-621.

Kitao, M., Lei, T.T., Koike, T., Tobita, H., Maruyama, Y., 2000. Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. Plant Cell Environ. 23, 81-89.

Kitao, M., Lei, T.T., Koike, T., Tobita, H., Maruyama, Y., 2006. Tradeoff between shade adaptation and mitigation of photoinhibition in leaves of *Quercus mongolica* and *Acer mono* acclimated to deep shade. Tree Physiol. 26, 441-448.

Krall, J.P., Edwards, G.E., 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. Physiol. Plant. 86, 180-187.

Liakopoulos, G., Nikolopoulos, D., Klouvatou, A., Vekkos, K., Manetas, Y., Karabourniotis, G., 2006. The photoprotective role of epidermal anthocyanins and surface pubescence in young leaves of grapevine (*Vitis vinifera*). Ann. Bot. 98, 257-265.

Liu, N., Peng, C.-L., Lin, Z.-F., Lin, G.-Z., Pan, X.-P., 2007. Regulation of light energy utilization and distribution of photosynthesis in five subtropical woody plants. J. Integr. Plant Biol. 49, 351-361.

Müller, P., Li, X.-P., Niyogi, K.K., 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125, 1558-1566.

Murata, N., Allakhverdiev, S.I., Nishiyama, Y., 2012. The mechanism of photoinhibition in vivo: re-evaluation of the roles of catalase, α-tocopherol, non-photochemical quenching, and electron transport. Biochim. Biophy. Acta 1817, 1127-1133.

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y.I., Neukermans, J., Marquez-Garcia, B., Queval, G., Foyer, C.H., 2012. Glutathione in plants: an integrated overview.

Plant Cell Environ. 35, 454-484.

Peng, S., Wang, B., 1994. Forest succession of Dinghushan, Guangdong, China. Chin. J Bot. 7, 75-80.

Rehman, F., Khan, F.A., Badruddin, S.M.A., 2012. Role of phenolics in plant defense against insect herbivory, in: Khemani, L.D., Srivastava, M.M., Srivastava, S. (Eds.), Chemistry of Phytopotentials: Health, Energy and Environmental Perspectives. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 309-313.

Rice-Evans, C., Miller, N., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2, 152-159.

Saha, M., Hasan, S., Akter, R., Hossain, M., Alam, M., Alam, M., Mazumder, M., 2008. *In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. Bangla. J. Vet. Med. 6, 197-202.

Salgado, P.R., Favarin, J.L., Leandro, R.A., Lima Filho, O.F.d., 2008. Total phenol concentrations in coffee tree leaves during fruit development. Sci. Agric. 65, 354-359.

Silva, E.N., Silveira, J.A.G., Ribeiro, R.V., Vieira, S.A., 2015. Photoprotective function of energy dissipation by thermal processes and photorespiratory mechanisms in *Jatropha curcas* plants during different intensities of drought and after recovery. Environ. Experim. Bot. 110, 36-45.

Smirnoff, N., 2000. Ascorbate biosynthesis and function in photoprotection. Phil. Trans. Roy. Soc. London Ser. B Biol. Sci. 355, 1455-1464.

Takahashi, S., Badger, M.R., 2011. Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci. 16, 53-60.

Tucić, B., Vuleta, A., Jovanovic, S.M., 2009. Protective function of foliar anthocyanins: in situ experiments on a sun-exposed population of *Iris pumila* L. (Iridaceae). Pol. J. Ecol. 57, 779-783.

Valentini, R., Epron, D., Angelis, P.d., Matteucci, G., Dreyer, E., 1995. *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q. cerris* L.) leaves: diurnal cycles under different levels of water supply. Plant Cell Environ. 18, 631-640.

Voss, I., Sunil, B., Scheibe, R., Raghavendra, A., 2013. Emerging concept for the role of photorespiration as an important part of abiotic stress response. Plant Biol. 15, 713-722.

Wang, H., Cao, G., Prior, R.L., 1997. Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem. 45, 304-309.

Wellburn, A.R., 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307-313.

Young, A.J., 1991. The photoprotective role of carotenoids in higher plants. Physiol. Plant. 83, 702-708.

Zhang, K.-M., Yu, H.-J., Shi, K., Zhou, Y.-H., Yu, J.-Q., Xia, X.-J., 2010. Photoprotective roles of anthocyanins in *Begonia semperflorens*. Plant Sci. 179, 202-208.

Zhang, Q., Chen, Y., Song, L., Liu, N., Sun, L., Peng, C., 2012. Utilization of lightflecks by seedlings of five dominant tree species of different subtropical forest successional stages under low-light growth conditions. Tree Physiol. 32, 545-553.

Zhang, Q., Zhang, T.-J., Chow, W.S., Xie, X., Chen, Y.-J., Peng, C.-L., 2015. Photosynthetic characteristics and light energy conversions under different light environments in five tree species occupying dominant status at different stages of subtropical forest succession. Funct. Plant Biol. 42, 609-619.

Zhang, T.-J., Chow, W.S., Liu, X.-T., Zhang, P., Liu, N., Peng, C.-L., 2016. A magic red coat on the surface of young leaves: anthocyanins distributed in trichome layer protect *Castanopsis fissa* leaves from photoinhibition. Tree Physiol. 36, 1296-1306.

Zhu, H., Zhang, T.J., Zhang, P., Peng, C.L., 2016. Pigment patterns and photoprotection of anthocyanins in the young leaves of four dominant subtropical forest tree species in two successional stages under contrasting light conditions. Tree Physiol. 36, 1092-1104.

Zhu, H., Zhang, T.-J., Zheng, J., Huang, X.-D., Yu, Z.-C., Peng, C.-L., Chow, W.S.,

2017. Anthocyanins function as a light attenuator to compensate for insufficient photoprotection mediated by nonphotochemical quenching in young leaves of *Acmena acuminatissima* in winter. Photosynthetica, DOI: 10.1007/s11099-017-0740-1.

Zhu, S.D., Song, J.J., Li, R.H., Ye, Q., 2013. Plant hydraulics and photosynthesis of 34 woody species from different successional stages of subtropical forests. Plant Cell Environ. 36, 879-891.

Table 1. Length and width and relative maturity (in brackets) of leaves of six species used for experimental study.

	FL		LL	
	Length (cm)	Width (cm)	Length (cm)	Width (cm)
Castanopsis fissa				
1st	7.1±0.3 (26%)	2.2±0.1 (23%)	7.1±0.7 (24%)	2.0±0.2 (16%)
2nd	11.8±0.5 (44%)	4.3±0.4 (44%)	16.8±2.2 (56%)	5.2±0.6 (44%)
ML	27.0±1.6 (100%)	9.7±1.0 (100%)	29.8±2.7 (100%)	12.0±0.9 (100%)
Castanopsis chinenesis				
1st	7.7±0.5 (60%)	1.8±0.1 (62%)	8.4±0.1 (59%)	1.8±0.1 (61%)
2nd	11.3±0.9 (87%)	2.3±0.1 (80%)	11.0±0.9 (77%)	2.5±0.2 (85%)
ML	12.9±0.9 (100%)	2.9±0.2 (100%)	14.3±0.6 (100%)	2.9±0.2 (100%)
Schima superba				
1st	6.4±0.2 (66%)	2.0±0.1 (65%)	7.3±0.4 (65%)	2.2±0.1 (58%)
2nd	8.1±0.6 (83%)	2.8±0.3 (90%)	8.5±0.7 (75%)	2.7±0.3 (73%)
ML	9.8±0.5 (100%)	3.1±0.1 (100%)	11.3±0.5 (100%)	3.7±0.3 (100%)
Machilus chinensis				
1st	7.4±0.1 (60%)	1.6±0.1 (64%)	9.6±0.3 (65%)	2.0±0.2 (68%)
2nd	8.5±0.2 (69%)	2.1±0.1 (84%)	12.1±0.5 (82%)	2.6±0.1 (91%)
ML	12.3±0.8 (100%)	2.4±0.1 (100%)	14.9±0.1 (100%)	2.9±0.1 (100%)
Cryptocarya chinensis				
1st	4.5±0.3 (47%)	2.3±0.1 (48%)	5.6±0.4 (53%)	2.2±0.2 (42%)
2nd	6.1±0.8 (64%)	3.3±0.2 (69%)	7.9±0.5 (75%)	3.7±0.1 (69%)
ML	9.6±0.4 (100%)	4.8±0.1 (100%)	10.5±0.5 (100%)	5.4±0.3 (100%)
Cryptocarya concinna				
1st	6.0±0.3 (61%)	2.0±0.1 (60%)	6.2±0.4 (63%)	2.2±0.1 (62%)
2nd	7.8±0.4 (80%)	2.9±0.1 (85%)	7.9±0.9 (80%)	2.8±0.1 (80%)
ML	9.7±0.4 (100%)	3.4±0.3 (100%)	9.9±0.8 (100%)	3.5±0.1 (100%)

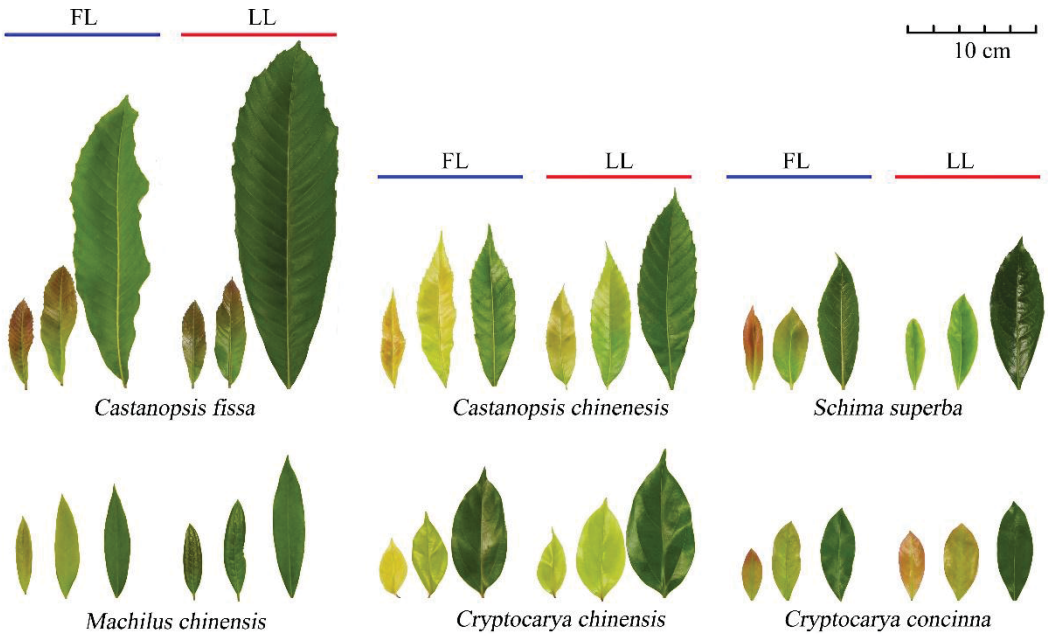

Figure 1. Appearances of young and mature leaves of six tree species grown under 100% (FL) and 30% full light (LL). *Castanopsis fissa*, *Castanopsis chinensis* and *Schima superba* are dominant in the mid-successional stages of subtropical forest, and *Machilus chinensis*, *Cryptocarya chinensis* and *Cryptocarya concinna* are dominant in the late-successional stages. In each light condition, the 1st 2nd and mature leaves are shown from left to right, respectively.

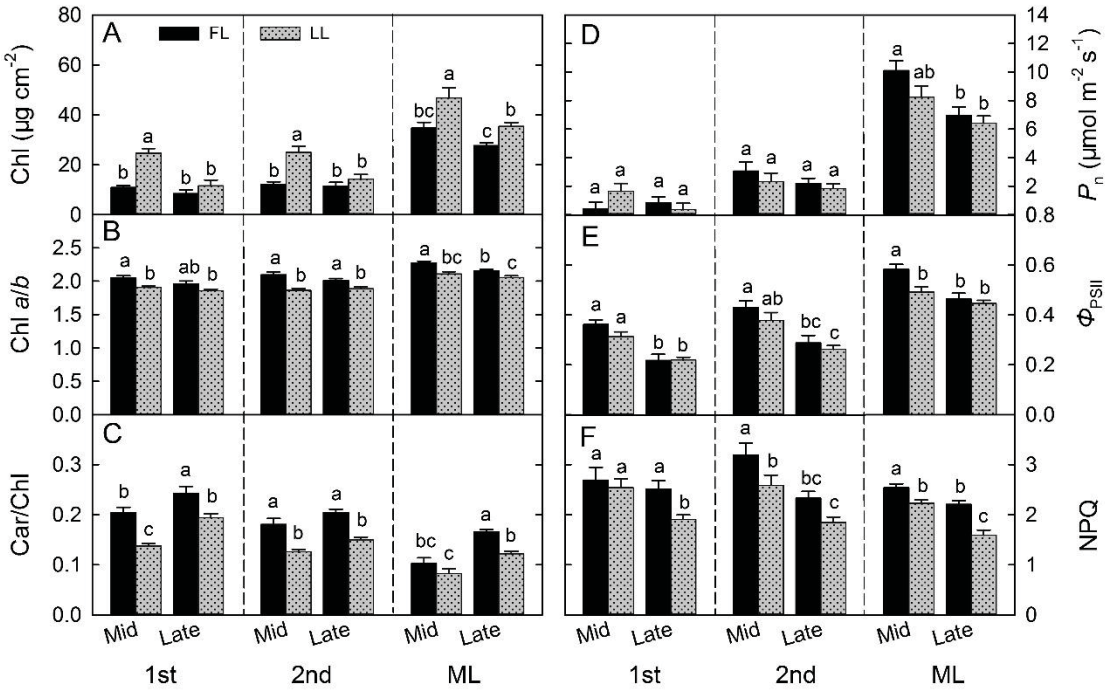

Figure 2. Total Chlorophylls (Chl, A), Chl *a*/*b* (B) and carotenoids/chlorophylls (Car/Chl, C), net photosynthesis (P_n, D), effective photochemical yield (Φ_{PSII}, E), non-photochemical quenching (NPQ, F) in young and mature leaves of two successional tree groups. Data are mean ± SE (n = 15). Different letters above bars indicate statistical significance ($P < 0.05$). FL = 100% full sunlight; LL = 30% full sunlight; 1st, 2nd and ML are the first, second young and mature leaves, respectively; Mid = mid-successional tree group, including *Castanopsis fissa*, *Castanopsis chinensis* and *Schima superba*; Late = late-successional species tree group, including *Machilus chinensis*, *Cryptocarya chinensis* and *Cryptocarya concinna*.

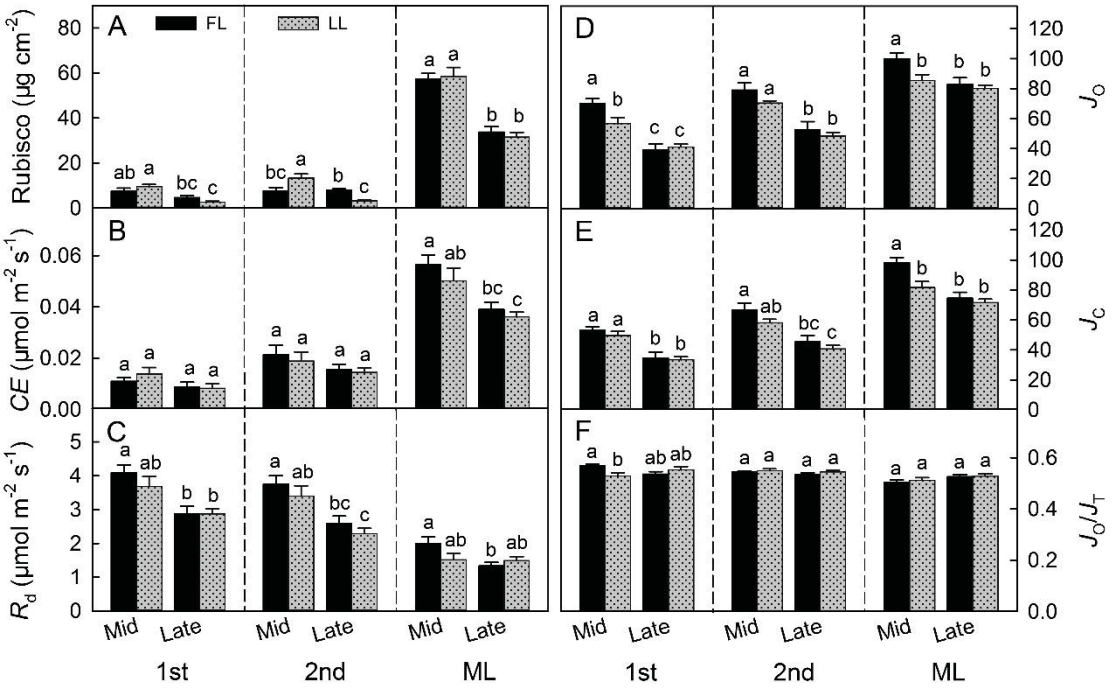

Figure 3. Rubisco large subunit content (A), carboxylation efficiency (*CE*, B), dark respiration (R_d, C), electron transport to Rubisco-dependent oxygenation (J_O, D) carboxylation (J_C, E) and proportion of photosynthetic electron transport to photorespiration (J_O/J_T, F) in young and mature leaves of two successional tree groups. Data are mean ± SE (n = 12-15). Different letters above bars indicate statistical significance ($P < 0.05$). FL = 100% full light; LL = 30% full light; 1st, 2nd and ML are the first, second young and mature leaves, respectively; Mid = mid-successional tree group, including *Castanopsis fissa*, *Castanopsis chinensis* and *Schima superba*; Late = late-successional species tree group, including *Machilus chinensis*, *Cryptocarya chinensis* and *Cryptocarya concinna*.

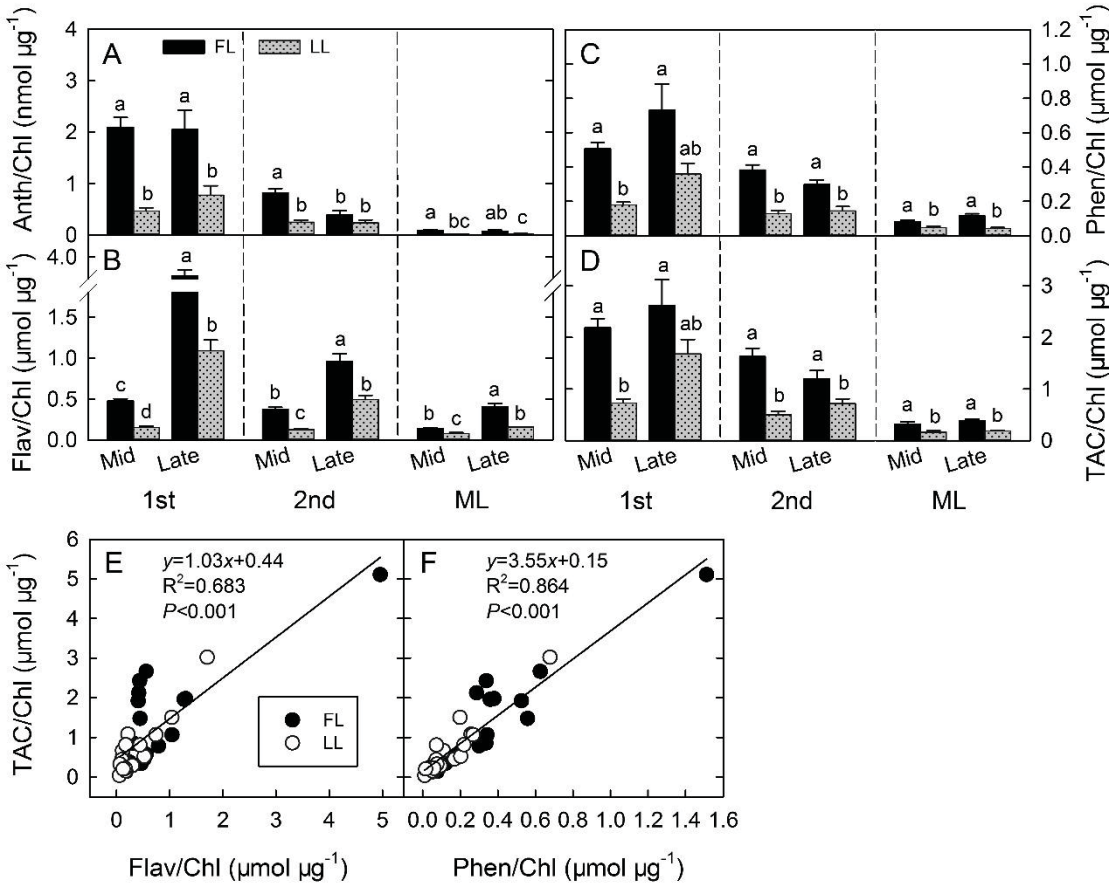

Figure 4. Ratios of anthocyanins/chlorophylls (Anth/Chl, A), flavonoids/chlorophylls (Flav/Chl, B), phenols/chlorophylls (Phen/Chl, C), and total antioxidant capacity/chlorophylls (TAC/Chl, D) in young and mature leaves of two successional tree groups, and correlations between TAC/Chl and Flav/Chl (E), and Phen/Chl (F) in various leaves of six species. Data are mean ± SE (n = 15). Different letters above bars indicate statistical significance ($P < 0.05$). FL = 100% full light; LL = 30% full light; 1st, 2nd and ML are the first, second young and mature leaves, respectively; Mid = mid-successional tree group, including *Castanopsis fissa*, *Castanopsis chinensis* and *Schima superba*; Late = late-successional species tree group, including *Machilus chinensis*, *Cryptocarya chinensis* and *Cryptocarya concinna*.

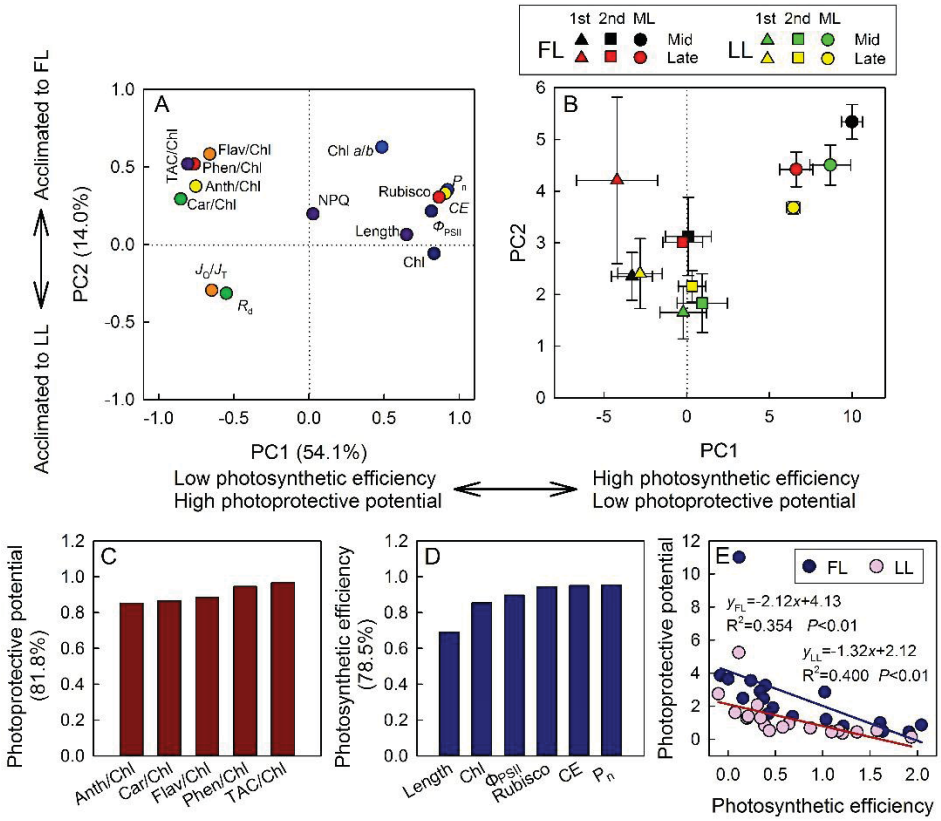

Figure 5. Principal component analysis for 15 functional traits (A) and loadings of young and mature leaves of six species categorized into mid- and late-successional groups on the first and second axes (B); secondary principal component analysis for 5 photoprotective traits (C) and 6 photosynthetically associated traits (D); correlations between photoprotective potential and photosynthetic efficiency (E) in young and mature leaves of six species. 15 functional traits are leaf length (Length), chlorophyll content (Chl), Chl *a*/*b*, anthocyanins/chlorophylls (Anth/Chl), flavonoids/chlorophylls (Flav/Chl), phenols/chlorophylls (Phen/Chl), total antioxidant capacity/chlorophylls (TAC/Chl), net photosynthesis (P_n), Rubisco large chain content (Rubisco), carboxylation efficiency (*CE*), dark respiration (R_d), effective photochemical yield (Φ_{PSII}), proportion of photosynthetic electron transport to photorespiration (J_O/J_T); NPQ, non-photochemical quenching. FL=100% full light; LL = 30% full light; 1st, 2nd and ML are the first, second young and mature leaves, respectively; Mid = mid-successional tree group, including *Castanopsis fissa*, *Castanopsis chinensis* and *Schima superba*; Late = late-successional species tree group, including *Machilus chinensis*, *Cryptocarya chinensis* and *Cryptocarya concinna*.